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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PRODUCTION OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR AND DNA ENCODING SAME

(57) Abstract

DNA isolates coding for a vascular endothelial cell growth factor may be used to produce the protein via recombinant expression systems. Such protein is useful therapeutically to treat conditions in which a selective action on the vascular endothelial cells, in the absence of excessive connective tissue proliferation, is desirable.

whole document reviewed ✓
not helpful

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid sequence comprising a sequence that encodes a vascular endothelial cell growth factor having a molecular weight of about 45,000 daltons under non-reducing conditions and about 23,000 under reducing conditions as measured by SDS-PAGE.

2. The sequence of claim 1 wherein the growth factor is bovine.

3. The sequence of claim 1 wherein the growth factor is human.

4. The sequence of claim 1 that is a DNA sequence.

5. An isolated DNA sequence comprising a sequence that hybridizes to the DNA sequence:

5'-CCTATGGCTGAAGGCGGCCAGAAGCCTCACGAAGTGGTGAAGTTCA-TGGACGTGTATCA-3' when incubated therewith at 42°C in 20% formamide, 5 x SSC, 50 mM sodium phosphate pH 6.8, 0.1% sodium pyrophosphate, 5 x Denhardt's solution, and 50 μg/ml salmon sperm DNA, and washed with 2 x SSC, 0.1% SDS at 42°C, wherein said isolated sequence contains at least about ten nucleotides.

6. The sequence of claim 5 that contains at least about twenty nucleotides.

7. The sequence of claim 5 that hybridizes to the DNA sequence of Figure 2 when incubated therewith at 42°C in 20% formamide, 5 x SSC, 50 mM sodium phosphate pH 6.8, 0.1% sodium pyrophosphate, 5 x Denhardt's solution, and 50 μg/ml salmon sperm DNA, and washed with 2 x SSC, 0.1% SDS at 42°C, wherein said isolated sequence contains at least about ten nucleotides.

8. The sequence of claim 7 that contains at least about twenty nucleotides.

9. The sequence of claim 8 that contains at least about thirty nucleotides.

10. An isolated DNA sequence comprising a sequence that hybridizes to the DNA sequence of Fig. 2 when incubated therewith at 42°C in 50% formamide, 5 x SSC, 50 mM sodium phosphate pH 6.8, 0.1% sodium pyrophosphate, 5 x Denhardt's solution, and 50 μg/ml salmon sperm DNA, and washed with 0.2 x SSC, 0.1% SDS at 42°C, wherein said isolated sequence contains at least about ten nucleotides.

11. The sequence of claim 10 that contains at least about twenty nucleotides.

12. The sequence of claim 10 that contains at least about thirty nucleotides.

13. The nucleic acid sequence of claim 5 further comprising a promoter operably linked to said nucleic acid sequence.

14. The nucleic acid sequence of claim 10 further comprising a promoter operably linked to said nuclec acid sequence.

15. An expression vector comprising the nucleic acid sequence of claim 5 operably linked to control sequences recognized by a host transformed by the vector.

16. An expression vector comprising the nucleic acid sequence of claim 10 operably linked to control sequences recognized by a host transformed by the vector.

17. A host cell transformed with the expression vector of claim 15.

18. The host cell of claim 17 wherein the cell is eukaryotic.

19. The host cell of claim 17 wherein the cell is prokaryotic.

20. A host cell transformed with the expression vector of claim 16.

21. An isolated DNA sequence comprising a DNA sequence encoding a vascular endothelial cell growth factor having an amino acid sequence sufficiently duplicative of that of vascular endothelial cell growth factor to allow it to possess one or both of the biological properties of (a) promoting growth selectively of vascular endothelial cells but not bovine corneal endothelial cells, lens epithelial cells, adrenal cortex cells, BHK-21 fibroblasts, or keratinocytes, or (b) cross-reacting immunologically with an antibody raised against at least one epitope of the corresponding native protein.

22. The sequence of claim 21 that is a cDNA sequence.

23. The sequence of claim 21 further comprising a signal sequence-encoding region N-terminal to the cell growth factor-encoding sequence.

24. The sequence of claim 23 wherein the signal sequence is recognized by mammalian cells.

25. The sequence of claim 21 that is a genomic sequence.

26. The sequence of claim 21 that is covalently bound to a detectable moiety.

27. An expression vector comprising the DNA sequence of claim 21 operably linked to control sequences recongized by a host transformed with the vector.

28. The vector of claim 27 that is a plasmid.

29. A host cell transformed with the expression vector of claim 27.

30. The host cell of claim 29 that is eukaryotic.

31. The host cell of claim 30 that is mammalian.

32. A method of producing a vascular endothelial cell growth factor, which method comprises culturing the cells of claim 29 to express the growth factor in the host cell culture.

33. The method of claim 32 further comprising the step of recovering the growth factor from the host cell culture.

34. The method of claim 33 wherein the growth factor is recovered from the host cell culture medium.

36. Vascular endothelial cell growth factor that is unaccompanied by associated native glycosylation, that has at least about 80% homology with the amino acid sequence of the mature protein shown in Fig. 2, and that possesses one or both of the biological properties of (a) promoting growth selectively of vascular endothelial cells but not bovine corneal endothelial cells, lens epithelial cells, adrenal cortex cells, BHK-21 fibroblasts, or keratinocytes, or (b) cross-reacting immunologically with an antibody raised against at least one epitope of the corresponding native protein.

37. The cell growth factor of claim 36 that is of bovine origin.

38. The cell growth factor of claim 36 that is of human origin.

39. Vascular endothelial cell growth factor that is completely free of source proteins.

40. The cell growth factor of claim 39 that is of bovine origin.

41. The cell growth factor of claim 39 that is of human origin.

42. A pharmaceutical composition useful for promotion of vascular endothelial cell growth comprising a therapeutically effective amount of the growth factor of claim 36 in a pharmaceutically acceptable carrier.

43. The composition of claim 42 further comprising another cell growth factor.

44. The composition of claim 42 that is isotonic.

45. The composition of claim 42 that is sterile filtered.

46. A method for treating trauma affecting the vascular endothelium comprising administering to an animal or human suffering from said trauma an effective amount of the composition of claim 42.

47. A method for treating trauma affecting the vascular endothelium comprising administering to an animal or human suffering from said trauma an effective amount of the composition of claim 43.

48. The method of claim 46 further comprising administering to said animal or human an effective amount of another cell growth factor.

49. The method of claim 46 wherein the trauma is diabetic ulcers or a wound of the blood vessels or heart.

50. The method of claim 46 wherein a human is treated.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/02585

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 N 15/16, C 12 P 21/02, C 07 K 13/00, A 61 K 37/24, C 12 N 5/10, C 12 N 1/21, // C 12 N 15/62

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 N, C 12 P, C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,P	Biochemical and Biophysical Research Communications, vol. 161, no. 2, 15 June 1989, Academic Press, Inc., N. Ferrara et al.: "Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells", pages 851-858, see the whole article (cited in the application) --	36-37,39-40
X	Science, vol. 219, 1983, D.R. Senger et al.: "Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid", pages 983-985, see the whole article --	36,39

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
8th August 1990	06. 09. 90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	R.J. Eernisse

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Science, vol. 246, no. 4935, 8 December 1989, (Washington, DC., US), D.W. Leung et al.: "Vascular endothelial growth factor is a secreted angiogenic mitogen", pages 1306-1309, see the whole article (cited in the application)	1-41
X	Science, vol. 246, no. 4935, 8 December 1989, (Washington, DC., US), P.J. Keck et al.: "Vascular permeability factor, an endothelial cell mitogen related to PDGF", pages 1309-1312, see the whole article (cited in the application)	1-41
P,X	Biochemical and Biophysical Research Communications, vol. 165, no. 3, 29 December 1989, Academic Press, Inc., E. Tischer et al.: "Vascular endothelial growth factor: A new member of the platelet-derived growth factor gene family", pages 1198-1206, see the whole article	1-2,4-37, 39-40

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers ...*..... because they relate to subject matter not required to be searched by this Authority, namely:

* See PCT-Rule 39.1(IV): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.